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(54) Title: FATTY ACID ANALOGUES FOR THE TREATMENT OF INFLAMMATORY AND AUTOIMMUNE DISORDERS

(57) Abstract: The present invention relates to fatty acid analogues of the general formula I: R_1- $[X_i$ - $CH_2]_n$ - $COOR_2$; wherein R_1 is ; a C_1-C_{24} alkene with one or more double bonds and/or with one or more triple bonds, and/or; a C_1-C_{24} alkyne, and/or; a C_{1-C_624} alkyl, or a C_1-C_{24} alkyl substituted in one or several positions with one or more compounds selected from the group comprising fluoride, chloride, hydroxy, C_1-C_4 alkoxy, C_1-C_4 alkylthio, C_2-C_5 acyloxy or C_1-C_4 alkyl, and; whereinR2 represents hydrogen or C_1-C_4 alkyl, and; wherein *n* is an integer from 1 to 12, and; wherein *i* is an old number and indicates the position relative to $COOR_2$, and; wherein X_i independent of each other are selected from the group comprising, O, S, SO, SO_2, Se and CH_2, and; with the proviso that at least one of the X_i is not CH_2; which can be used for the treatment and/or prevention of inflammatory disorders. Further, the invention relates to methods for enhancing the endogenous production of interleukin-10 (IL-10) and suppressing the production of interleukin-2 in mammalian cells or tissues. The invention also relates to a method for inhibiting the proliferation of stimulated peripheral mononuclear cells.

FATTY ACID ANALOGUES FOR THE TREATMENT OF INFLAMMATORY AND AUTOIMMUNE DISORDERS.

The present invention relates to fatty acid analogues that can be used for the treatment and/or prevention inflammatory disorders. Further, the invention also relates to methods for enhancing the endogenous production of interleukin-10 (IL-10) and suppressing the production of interleukin-2 in mammalian cells or tissues. The invention also relates to a method for inhibiting the proliferation of stimulated peripheral mononuclear cells.

BACKGROUND OF THE INVENTION

Interleukins, interferons, colony stimulating factors and TNFα are examples of a group of diverse multi-functional proteins called cytokines. Cytokines are a class of secreted soluble proteins normally present in very low concentration in a variety of cells. Lymphoid, inflammatory hemopoietic and other cells such as connective tissue cells (e.g. fibroblasts, osteoblasts) secrete a variety of cytokines which regulate the immune, inflammatory, repair and acute phase responses by controlling cell proliferation, differentiation and effector functions. The effects of cytokines are mediated through binding to high affinity receptors on specific cell types.

An important cytokine is IL-10, a 35-40 kDa peptide produced by helper T-cells, B-cells, monocytes, macrophages and other cell types. In vitro, IL-10 has demonstrated immunosuppressive properties as evidenced by its ability to

suppress cytokine production including IL-1 and TNFα.

IL-10 also inhibits activation of other inflammatory cytokines, and therefore has potent anti-inflammatory activity.

It has been of recent interest to administer IL-10 in the treatment of certain conditions characterized by excessive IL-1 and TNFα production. Such diseases or conditions include loosening of prosthetic joint implants, inflammation, diabetes, cancer, graft versus host diseases, viral, fungal and bacterial infections, lipopolysaccharide endotoxin shock, diseases of depressed bone marrow function, thrombocytopenia, osteoporosis, spondyloarthropathies, Paget's disease, inflammatory bowel disease, arthritis, osteoarthritis, autoimmune diseases such as rheumatoid arthritis, systemic lupus erythematosus, and connective tissue diseases.

For example, purified IL-10 has been shown in vitro to suppress certain types of viral infections. U.S. Pat. No. 5,665,345 discloses a method for inhibiting replication of the human immunodeficiency virus, retro-viruses, and Kaposi sarcoma in human cells by administering IL-10.

IL-10 has also been suggested for use in the treatment of certain cancers. U.S. Pat. No. 5,570,190 discloses administering exogenous IL-10 to treat mammals suffering from acute myelogenous leukemia and acute lymphocytic leukemia. IL-10 is said to be administered either in the purified or recombinant form and is believed to inhibit the proliferation of acute leukemia blast cells.

Similarly, IL-10 was shown to inhibit bone marrow metastasis in severe combined immunodeficient mice.

The above conventional approaches to treating conditions characterized by excessive IL-1 and TNFα production have been limited to administering exogenous purified or recombinant IL-10 intravenously. Since IL-10 is a protein, it is difficult to infuse intravenously into a mammal because proteins often leach out of solution and bind to the plastic or glass used in intravenous administration sets. Also, proteins are often incompatible and precipitate when mixed with physiological solutions such as dextrose or saline. In addition, oral and topical routes are unavailable for IL-10 administration. The oral route is unavailable because protein is degraded in the gastrointestinal tract.

None of the above approaches suggests enhancing endogenous IL-10 production in mammals for prophylaxis and treatment of diseases or conditions.

Further, it is known that IL-10 is a powerful deactivator of macrophages and T cells, and inadequate production has been implicated in various autoimmune and inflammatory disorders.

The present study shows that TTA enhance both LPS and PHA stimulated IL-10, and suppress PHA stimulated IL-2 production in PBMC from healthy blood donors. This may have several implications. First, these findings suggest a marked anti-inflammatory net effect of TTA by both enhancing the release of the anti-inflammatory cytokine IL-10 and by suppressing the release of the inflammatory cytokine IL-2. Second, our findings suggest that TTA may modulate both monocyte (i.e. LPS stimulation) and lymphocyte activation (i.e. PHA stimulation). Finally, the *in vitro* effect of TTA on activated PBMC from healthy blood donors may reflect the situation in various patient populations characterized by enhanced inflammatory

activation *in vivo*. In fact, *ex vivo* activated PBMC from healthy controls, may represent the relevant target cells for therapeutically intervention *in vivo* in various inflammatory disorders.

DETAILED DESCRIPTION OF THE INVENTION

The present patent application discloses that a preferable compound of the invention, i.e the thia-substituted fatty acid tetradecylthioacetic acid (TTA) modulates the release of inflammatory (i.e. IL-2, IL-1β and TNF-α) and anti-inflammatory (i.e. IL-10) cytokines in the cultured cell line PBMC.

More specifically the present invention discloses that TTA markedly suppresses the PHA stimulated release of IL-2, and also enhances the PHA stimulated release of IL-10.

These two effects adds up to a profound anti-inflammatory effect, and it is thus anticipated that the compounds of the present invention hold promises as interesting compounds for the treatment and/or prevention of disorders related to inflammation.

The present invention thus relates to the use of fatty acid analogues of the general formula (I):

$$R_1\text{-}\ [x_i\ -\ CH_2]_n\ -\ COOR_2 \qquad (I)$$

- wherein R_1 is;
  - a C_1-C_{24} alkene with one or more double bonds and/or with one or more triple bonds, or
  - a C_1-C_{24} alkyne, or
  - a C_1-C_{24} alkyl, or a C_1-C_{24} alkyl substituted in one or several positions with one or more compounds selected from the group comprising

fluoride, chloride, hydroxy, C_1-C_4 alkoxy, C_1-C_4 alkylthio, C_2-C_5 acyloxy or C_1-C_4 alkyl, and

- wherein R2 represents hydrogen or C_1-C_4 alkyl, and

- wherein *n* is an integer from 1 to 12, and

- wherein *i* is an odd number and indicates the position relative to $COOR_2$, and

- wherein X_i independent of each other are selected from the group comprising O, S, SO, SO_2, Se and CH_2, and

- with the proviso that at least one of the X_i is not CH_2,

- with the proviso that if R1 is an alkyne, then one of the carbon-carbon triple bonds is positioned between the (ω-1) carbon and the (ω-2) carbon, or between the (ω-2) carbon and the (ω-3) carbon, or between the (ω-3) carbon and the (ω-4) carbon, and

- with the proviso that if R1 is an alkene, then one of the carbon-carbon triple bonds is positioned between the (ω-1) carbon and the (ω-2) carbon, or between the (ω-2) carbon and the (ω-3) carbon,

or a salt, prodrug or complex thereof, for the preparation of a pharmaceutical composition for the treatment and/or prevention of inflammatory disorders.

More specifically, the invention relates to methods for enhancing the endogenous production of interleukin-10 (IL-10) and suppressing the production of interleukin-2 in mammalian cells or tissues.

The invention also relates to a method for inhibiting the proliferation of stimulated peripheral mononuclear cells

Presently preferred embodiments of the present invention relates to the compounds tetradecylthioacetic acid (TTA) and tetradecylselenoacetic acid (TSA).

FIGURE LEGENDS

Figure 1 shows the effect of different concentrations of TTA on proliferation of PBMC.

Figure 2 shows the effect of various concentrations of TTA on the release of IL-10 (A), IL-2 (B), TNFα (C) and IL-1β (D) in PBMC supernatants.

Figure 3 shows the effect of TNFα (10 ng/mL) alone or in combination with different concentrations of TTA on the release of IL-10 (A) and IL-1β (B) in PBMC supernatants.

Figure 4. The effect of IL-2 (10 ng/mL) and anti-IL-10 (5 µg/mL) on the TTA-mediated inhibition of PHA stimulated PBMC proliferation.

ADMINISTRATION OF THE COMPOUNDS OF THE PRESENT INVENTION

As a pharmaceutical medicament the compounds of the present invention may be administered directly to the mammal by any suitable technique, including parenterally, intranasally, orally, or by absorption through the skin. They can be administered locally or systemically. The specific route of administration of each agent will depend, e.g., on the medical history of the mammal.

In addition, the compounds of the present invention are

appropriately administered in combination with other treatments for combating or preventing inflammatory and autoimmune disorders.

The invention will be more fully understood by reference to the following examples. They should not, however, be construed as limiting the scope of the invention.

EXPERIMENTAL SECTION

Example 1. Preparation and characterisation of the compounds

The synthesis of 3-substituted fatty acid analogues

The compounds used according to the present invention wherein the substituent $X_{i=3}$ is a sulphur atom or selenium atom may be prepared according to the following general procedure:

X is a sulphur atom:
The thio-substituted compound used according to the present invention may be prepared by the general procedure indicated below:

$$\text{Alkyl-Hal} + \text{HS-CH}_2\text{COOR} \xrightarrow{\text{Base}} \text{Alkyl-S-CH}_2\text{-COOR}$$

The sulphur-compound, namely, tetradecylthioaceticacid (TTA), $(CH_3-(CH_2)_{13}-S-CH_2-COOH$ was prepared as shown in EP-345.038.

X is a selenium atom:
the seleno-substituted compound used according to the present invention may be prepared by the following general procedure

1. Alkyl-Hal + KSeCN $\Rightarrow$ Alkyl-SeCN...

2. Alkyl-SeCN + BH_4^- ⇒ Alkyl-Se^-

3. Alkyl-Se^- + O_2 ⇒ Alkyl-Se-Se-Alkyl

This compound was purified by carefully crystallisation from ethanol or methanol.

4. Alkyl-Se-Se-Alkyl $\xrightarrow{BH_4^-}$ 2 Alkyl-Se^-

5. Alkyl-Se^- + Hal-CH_2-COOH ⇒ Alkyl-Se-CH_2 - COOH

The final compound, e.g. when alkyl is tetradecyl, $(CH_3-(CH_2)_{13}-Se-CH_2-COOH$ (tetradecylselenoacetic acid (TSA)) can be purified by crystallisation from diethyl ether and hexane.

Other compounds in accordance with the present invention can be synthesised as indicated in applicant's patent applications PCT/NO99/00135 and NO 20001123.

Example 2

Lymphocyte proliferation

Blood donor (n=5) peripheral blood mononuclear cells (PBMC) were obtained from heparinized blood by Isopaque-Ficoll (Lymphoprep, Nycomed Pharma AS, Oslo, Norway) gradient centrifugation within 1 hour after blood sampling. PBMC were resuspended in RPMI 1640 with 2 mM L-glutamine and 25 mM HEPES buffer (Gibco BRL, Paisley, UK) supplemented with 10% heat inactivated pooled human AB^+ serum (culture medium). The endotoxin level in culture medium, reagents and stimulants was < 10 pg/mL (Quantitative chromogenic limulus amebocyte lysate test, BioWhittaker, Inc., Walkerswille, MD).

PMNC (10^6cells/mL) were incubated in flat-bottomed, 96-well microtiter trays (200 µL/well; Costar, Cambridge, MA) in medium alone or with phytohemagglutinin (PHA; Murex

Diagnostics Ltd, Dartford, UK; final concentration 1:100) either alone or with different concentrations of TTA. Bovine serum albumin (BSA, Calbiochem, La Jolla, CA) was used as a negative control for TTA (vehicle). In some experiments neutralizing monoclonal anti-human interleukin (IL)-10 (final concentration 5 µg/mL; Endogen, Cambridge, MA) or recombinant human IL-2 (final concentration 10 ng/mL; R&D Systems, Minneapolis, MN) was also added to cell cultures before stimulation. After 48 hours, cells were pulsed with 1 µCi of ^{3}H-thymidine (Amersham International plc., Little Chalfont, UK), and 16 hours later cultures were harvested onto glass filter strips, using an automated multisampler harvester (Skatron, Lier, Norway). ^{3}H-thymidine incorporation was determined by liquid scintillation counting as counts per minute (cpm).

Results

While TTA had no effect on lymphocyte proliferation when given alone, TTA markedly suppressed PHA stimulated proliferation of PBMC in a dose-dependent manner (~60 reduction; Fig. 1). Such a suppressive effect was seen in all five blood donors. In contrast, no effect on PHA stimulated PBMC proliferation was when the vehicle (BSA) was given alone (Fig. 1).

Example 3

Release of cytokines in PBMC supernatants

PBMC (10^{6}cells/mL) were incubated in flat-bottomed, 96-well microtiter trays (200 µL/well, Costar) in medium alone (see above) or with PHA (final concentration 1:100), lipopolysaccharide (LPS) from *E. coli* O26:B6 (final concentration 10 ng/mL; Sigma, St. Louis, MO) or tumor necrosis factor (TNF)? (final concentration 10 ng/mL; R&D Systems) with or without different concentrations of TTA. BSA was used as a negative control for TTA (vehicle). Cell-

free supernatants were harvested after 20 hours and stored at -80°C.

Enzyme immunoassays (EIAs)

Concentration of cytokines in PBMC supernatants were analyzed by EIAs according to the manufacturer's description (IL-1β and IL-10: CLB, Amsterdam, Netherlands; IL-2: R&D Systems).

Statistical analysis

For evaluation of the effect of TTA (or BSA) on various parameters, the Paired-Samples T Test was used. P-values (two-sided) are considered significant when <0.05.

Results

The effect of TTA on cytokine levels in PBMC supernatants

As shown in figure 2, TTA alone had no effect on production of either of the cytokines IL-2, IL-1β, IL-10 and TNFα.

However, several significant findings were revealed when TTA were added to cell cultures in combination with PHA or LPS.

First, TTA markedly suppressed the PHA stimulated release of IL-2 in a dose-dependent manner (~75% reduction)(Fig. 2).

Second, in contrast to this suppressive effect, TTA in a dose-dependent manner markedly enhanced both LPS stimulated (~3-fold increase) and in particular PHA stimulated (~11-fold increase) release of the anti-inflammatory cytokine IL-10 (Fig. 2).

Third, in contrast to these pronounced effects on IL-2 and IL-10 levels, TTA had no or only modest effect on LPS stimulated release of TNFα and IL-1β (Fig. 2). There were no effects of the vehicle (BSA) on either PHA or LPS stimulated release of cytokines (Fig. 2).

In conclusion, TTA have several effects on LPS and in particular on PHA stimulated release of cytokines in PBMC favoring anti-inflammatory net effects.

The effect of TTA on TNFα stimulated release of cytokines in PBMC supernatants

Fatty acids have been reported to modulate various TNFα mediated effects. TNFα may induce the production of other cytokines such as IL-10 and IL-1β(11,12), and we therefore examined if TTA could modulate the TNFα induced release of these cytokines from PBMC in 5 healthy blood donors. Notably, while TTA had no effect on LPS stimulated release of TNF? (Fig. 2), TTA markedly enhanced the TNFα stimulated release of both IL-1β (~5-fold increase) and in particular of IL-10 (~11-fold increase)(Fig 3). These findings suggest that TTA can considerably enhance the TNFα stimulated release of cytokines from PBMC with particularly enhancing effect on the release of IL-10.

Example 4

Effect of IL-2 and anti-IL-10 on the TTA mediated inhibition of lymphocyte proliferation

IL-2 and IL-10 is known to enhance and inhibit lymphocyte proliferation, respectively. We therefore examined if the anti-proliferative effect of TTA on PHA stimulated PBMC proliferation was related to the TTA mediated effect on

these cytokines (see above). However, the addition of anti-IL-10 to cell cultures had no effect and IL-2 only a modest counteracting effect on the TTA mediated inhibition of lymphocyte proliferation (Fig. 4). Thus, it seems that the anti-proliferative and anti-inflammatory effects of TTA at least partly represent distinct biologic mechanisms.

Conclusions

As shown in the experimental section TTA has several effects on the release of cytokines from activated PBMC with a marked increase in IL-10 accompanied by a reduction in IL-2 levels. This favors anti-inflammatory net effects, and it is thus anticipated that the compounds of the present invention can be used to regulate inflammatory processes, and thus can be used as medicaments for the treatment and/or prevention of inflammatory disorders.

Further, we have shown that TTA potentates the cytokine stimulating effects of TNFα on these cells with particularly enhancing effect on the IL-10 levels.

Finally, TTA also significantly suppressed PBMC proliferation, and this anti-proliferative effect did not involve enhanced apoptosis and seems at least partly to be distinct from the anti-inflammatory effects of TTA.

Our findings suggest potent anti-inflammatory and anti-proliferative effects of TTA in activated PBMC in humans.

There are several disorders in which enhanced IL-10 and depressed IL-2 levels might be of therapeutically importance. This includes a wide range of immune mediated disorders such as rheumatoid arthritis, systemic vasculitis, systemic lupus erythematosus, systemic sclerosis, dermatomyositis, polymyositis, various

autoimmune endocrine disorders (e.g. thyroiditis and adrenalitis), various immune mediated neurological disorders (e.g. multiple sclerosis and myastenia gravis), various cardiovascular disorders (e.g. myocarditis, congestive heart failure, arteriosclerosis and stable and unstable angina, and Wegener's granulomatosis), inflammatory bowel diseases and Chron's colitis, nephritis, various inflammatory skin disorders (e.g. psoriasis, atopic dermatitis and food allergy) and acute and chronic allograft rejection after organ transplantation.

It is known that IL-10 is a powerful deactivator of macrophages and T cells, and inadequate production of IL-10 has been implicated in various autoimmune and inflammatory disorders. It is thus anticipated that the compound of the present invention can be used for the prevention and/or treatment of autoimmune and inflammatory disorders.

Autoimmune models of rheumatoid arthritis, thyroiditis, collagen-induced arthritis and experimental allergic encephalomyelitis all suggest a negatively regulatory role for IL-10 in limiting inflammation and immunopathology. Moreover, mice with a targeted disruption in the IL-10 gene spontaneously develop a generalized enterocolitis. In humans, Chron´s colitis and psoriasis may even be susceptible to treatment with systemically administered IL-10. Finally, IL-10 has recently also been found to have protective effects on the development of atherosclerosis and viral myocarditis in mice. Thus, treatment modalities which enhance IL-10 levels may be of great interest in the management of the above mentioned and other autoimmune and inflammatory disorders, and it is contemplated that the compounds of the present invention have such properties.

Further, we have shown that TTA markedly enhanced the TNFα induced IL-10 level, and such anti-inflammatory properties

if exploited therapeutically could potentially represent a protection against harmful effect of TNFα.

CLAIMS

1. Use of fatty acid analogues of the general formula (I):

$$R_1-[x_i-CH_2]_n-COOR_2 \qquad (I)$$

- wherein R_1 is;
  - a C_1-C_{24} alkene with one or more double bonds and/or with one or more triple bonds, or
  - a C_1-C_{24} alkyne, or
  - a C_1-C_{24} alkyl, or a C_1-C_{24} alkyl substituted in one or several positions with one or more compounds selected from the group comprising fluoride, chloride, hydroxy, C_1-C_4 alkoxy, C_1-C_4 alkylthio, C_2-C_5 acyloxy or C_1-C_4 alkyl, and

- wherein R2 represents hydrogen or C_1-C_4 alkyl, and

- wherein *n* is an integer from 1 to 12, and

- wherein *i* is an odd number and indicates the position relative to $COOR_2$, and

- wherein X_i independent of each other are selected from the group comprising O, S, SO, SO_2, Se and CH_2, and

- with the proviso that at least one of the X_i is not CH_2,

- with the proviso that if R1 is an alkyne, then one of the carbon-carbon triple bonds is positioned between the (ω-1) carbon and the (ω-2) carbon, or between the (ω-2) carbon and the (ω-3) carbon, or between the (ω-3) carbon and the (ω-4) carbon, and

- with the proviso that if R1 is an alkene, then one of the carbon-carbon triple bonds is positioned between the (ω-1) carbon and the (ω-2) carbon, or between the (ω-2) carbon and the (ω-3) carbon,

or a salt, prodrug or complex thereof, for the preparation of a pharmaceutical composition for the prevention and/or treatment of inflammatory disorders.

2. The use according to claim 1, wherein the compound is tetradecylthioacetic acid.

3. The use according to claim 1, wherein the compounds is tetradecylselenoacetic acid.

4. The use according to claim 1, wherein the compound is an alkene and contains only one double bond.

5. The use according to claim 1, wherein the compound is an alkyne and contains only one triple bond.

6. The use according to claim 1, wherein the inflammatory disorder is selected from the group comprising immune mediated disorders such as rheumatoid arthritis, systemic vasculitis, systemic lupus erythematosus, systemic sclerosis, dermatomyositis, polymyositis, various autoimmune endocrine disorders (e.g. thyroiditis and adrenalitis), various immune mediated neurological disorders (e.g. multiple sclerosis and myastenia gravis), various cardiovascular disorders (e.g. myocarditis, congestive heart failure, arteriosclerosis and stable and unstable angina, and Wegener's granulomatosis), inflammatory bowel diseases and Chron's colitis, nephritis, various inflammatory skin disorders (e.g. psoriasis, atopic dermatitis and food allergy) and acute and chronic

allograft rejection after organ transplantation.

7. A method for enhancing the endogenous production of interleukin-10 (IL-10) in mammalian cells or tissues, said method comprising the step of administering to a mammal in need thereof an effective amount of fatty acid analogues of the general formula (I):

$$R_1\text{-}\,[x_i\,-\,CH_2]_n\,-\,COOR_2 \qquad (I)$$

- wherein R_1 is;
  - a C_1-C_{24} alkene with one or more double bonds and/or with one or more triple bonds, or
  - a C_1-C_{24} alkyne, or
  - a C_1-C_{24} alkyl, or a C_1-C_{24} alkyl substituted in one or several positions with one or more compounds selected from the group comprising fluoride, chloride, hydroxy, C_1-C_4 alkoxy, C_1-C_4 alkylthio, C_2-C_5 acyloxy or C_1-C_4 alkyl, and

- wherein R2 represents hydrogen or C_1-C_4 alkyl, and

- wherein *n* is an integer from 1 to 12, and

- wherein *i* is an odd number and indicates the position relative to $COOR_2$, and

- wherein X_i independent of each other are selected from the group comprising O, S, SO, SO_2, Se and CH_2, and

- with the proviso that at least one of the X_i is not CH_2,

- with the proviso that if R1 is an alkyne, then one of the carbon-carbon triple bonds is positioned between the (ω-1) carbon and the (ω-2) carbon, or between the (ω-2) carbon and the (ω-3) carbon, or

between the (ω-3) carbon and the (ω-4) carbon, and

- with the proviso that if R1 is an alkene, then one of the carbon-carbon triple bonds is positioned between the (ω-1) carbon and the (ω-2) carbon, or between the (ω-2) carbon and the (ω-3) carbon,

or a salt, prodrug or complex thereof.

8. A method for suppression of the endogenous production of interleukin-2 (IL-2) in mammalian cells or tissues, said method comprising the step of administering to a mammal in need thereof an effective amount of fatty acid analogues of the general formula (I):

$$R_1\text{-}\ [x_i\ \text{-}\ CH_2]_n\ \text{-}\ COOR_2 \qquad (I)$$

- wherein R_1 is;
  - a C_1-C_{24} alkene with one or more double bonds and/or with one or more triple bonds, or
  - a C_1-C_{24} alkyne, or
  - a C_1-C_{24} alkyl, or a C_1-C_{24} alkyl substituted in one or several positions with one or more compounds selected from the group comprising fluoride, chloride, hydroxy, C_1-C_4 alkoxy, C_1-C_4 alkylthio, C_2-C_5 acyloxy or C_1-C_4 alkyl, and

- wherein R2 represents hydrogen or C_1-C_4 alkyl, and

- wherein *n* is an integer from 1 to 12, and

- wherein *i* is an odd number and indicates the position relative to $COOR_2$, and

- wherein X_i independent of each other are selected from the group comprising O, S, SO, SO_2, Se and CH_2, and

- with the proviso that at least one of the X_i is not CH_2,

- with the proviso that if R1 is an alkyne, then one of the carbon-carbon triple bonds is positioned between the (ω-1) carbon and the (ω-2) carbon, or between the (ω-2) carbon and the (ω-3) carbon, or between the (ω-3) carbon and the (ω-4) carbon, and

- with the proviso that if R1 is an alkene, then one of the carbon-carbon triple bonds is positioned between the (ω-1) carbon and the (ω-2) carbon, or between the (ω-2) carbon and the (ω-3) carbon,

or a salt, prodrug or complex thereof.

9. The method according to claim 7 or 8, wherein said mammalian cells or tissue are in a mammal.

10. The method according to claim 7 or 8, wherein the compound is tetradecylthioacetic acid.

11. The method according to claim 7 or 8, wherein the compound is tetradecylselenoacetic acid.

12. The method according to claim 7 or 8, wherein said mammal has developed or is susceptible to develop an autoimmune and/or inflammatory disorder.

13. The method according to claim 7 or 8, wherein said disorder is selected from the group comprising comprising immune mediated disorders such as rheumatoid arthritis, systemic vasculitis, systemic lupus erythematosus, systemic sclerosis, dermatomyositis, polymyositis, various autoimmune endocrine disorders (e.g. thyroiditis and adrenalitis), various immune mediated neurological disorders (e.g. multiple sclerosis and myastenia gravis), various cardiovascular disorders (e.g. myocarditis,

congestive heart failure, arteriosclerosis and stable and unstable angina, and Wegener's granulomatosis), inflammatory bowel diseases and Chron's colitis, nephritis, various inflammatory skin disorders (e.g. psoriasis, atopic dermatitis and food allergy) and acute and chronic allograft rejection after organ transplantation.

14. The method according to claim 7 or 8, wherein the compound is administered to a mammal characterized by excessive production and/or elevated levels of IL-1 and TNFα.

15. The method according to claim 7 or 8, wherein the compound is administered to a mammal characterized by inadequate production of IL-10.

16. Use of fatty acid analogues of the general formula (I):

$$R_1\text{-}\,[x_i\,\text{-}\,CH_2]_n\,\text{-}\,COOR_2 \qquad (I)$$

- wherein R_1 is;
  - a C_1-C_{24} alkene with one or more double bonds and/or with one or more triple bonds, or
  - a C_1-C_{24} alkyne, or
  - a C_1-C_{24} alkyl, or a C_1-C_{24} alkyl substituted in one or several positions with one or more compounds selected from the group comprising fluoride, chloride, hydroxy, C_1-C_4 alkoxy, C_1-C_4 alkylthio, C_2-C_5 acyloxy or C_1-C_4 alkyl, and

- wherein R2 represents hydrogen or C_1-C_4 alkyl, and

- wherein *n* is an integer from 1 to 12, and

- wherein *i* is an odd number and indicates the position relative to $COOR_2$, and

- wherein X_i independent of each other are selected from the group comprising O, S, SO, SO_2, Se and CH_2, and

- with the proviso that at least one of the X_i is not CH_2,

- with the proviso that if R1 is an alkyne, then one of the carbon-carbon triple bonds is positioned between the (ω-1) carbon and the (ω-2) carbon, or between the (ω-2) carbon and the (ω-3) carbon, or between the (ω-3) carbon and the (ω-4) carbon, and

- with the proviso that if R1 is an alkene, then one of the carbon-carbon triple bonds is positioned between the (ω-1) carbon and the (ω-2) carbon, or between the (ω-2) carbon and the (ω-3) carbon,

or a salt, prodrug or complex thereof, for the preparation of a pharmaceutical composition for the inhibition of proliferation of stimulated peripheral mononuclear cells (PBMC).

17. The use according to claim 16, wherein the cells are stimulated with a substance selected from the group comprising PHA, LPS and TNFα.

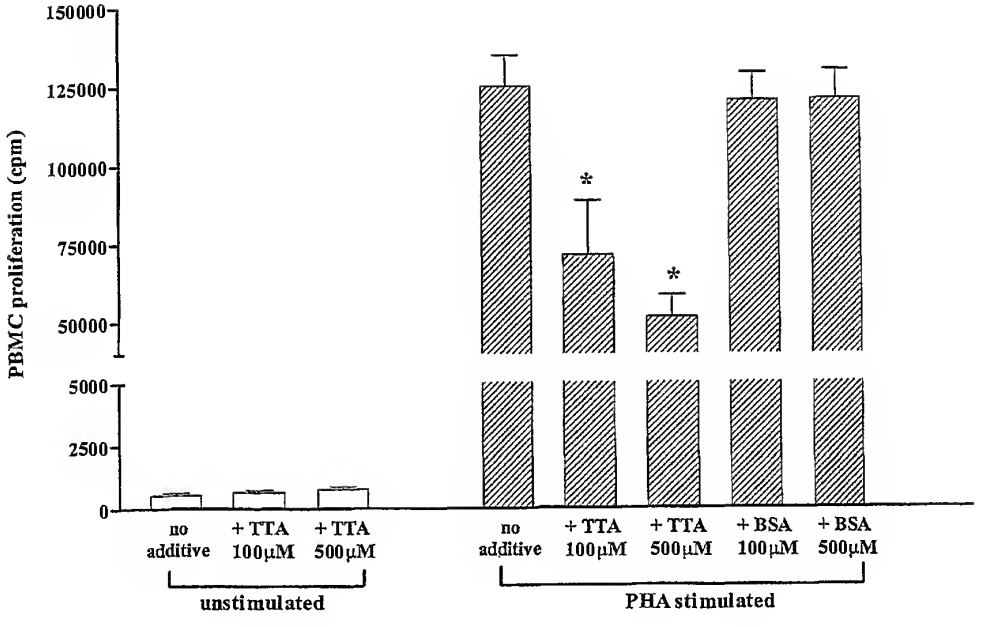
PBMC proliferation (cpm)
150000
125000
100000
75000
50000
5000
2500
0
*
*
no additive
+ TTA 100µM
+ TTA 500µM
no additive
+ TTA 100µM
+ TTA 500µM
+ BSA 100µM
+ BSA 500µM
unstimulated
PHA stimulated

FIG 1

A
IL-10 (pg/mL)
4000
3000
2000
1000
0
no additive
+TTA 100µM
+TTA 500µM
unstimulated
PHA stimulated
LPS stimulated
+BSA
+BSA 500µM
B
IL-2 (pg/mL)
1000
750
500
250
0
C
TNFα (pg/mL)
1600
1200
800
400
0
D
IL-1β (pg/mL)
10000
7500
5000
2500
0

FIG 2

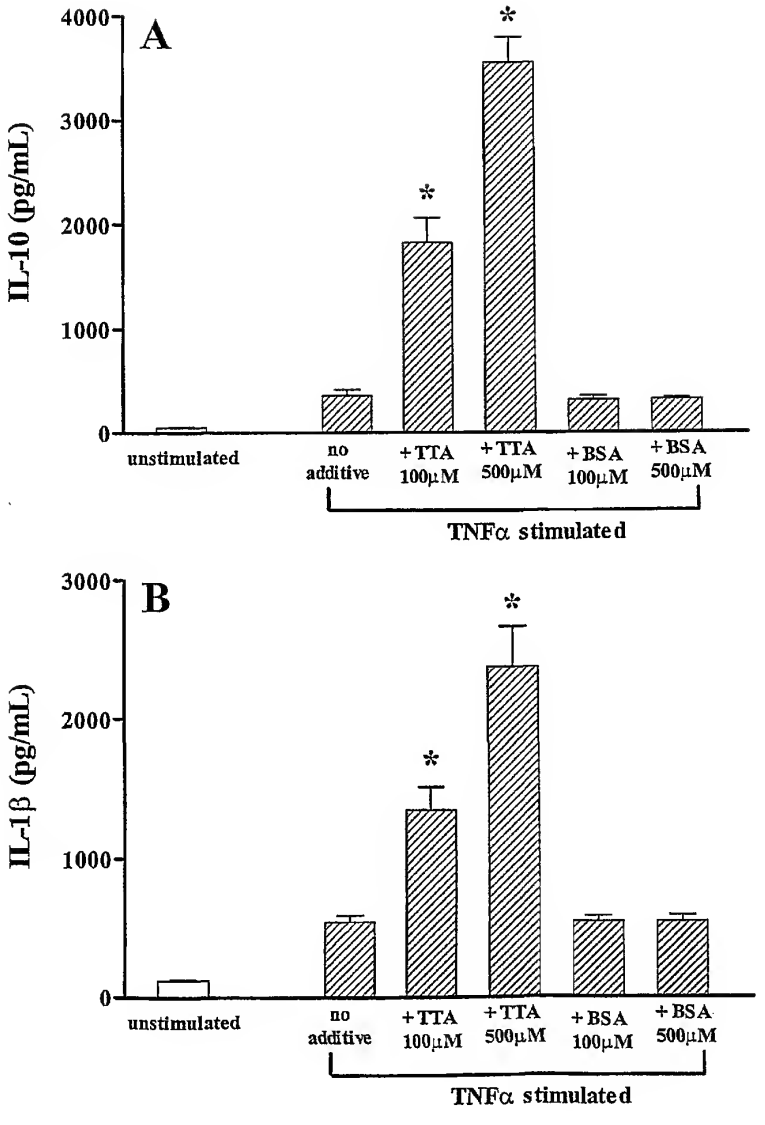
A
IL-10 (pg/mL)
4000
3000
2000
1000
0
*
*
unstimulated
no additive
+TTA 100μM
+TTA 500μM
+BSA 100μM
+BSA 500μM
TNFα stimulated
B
IL-1β (pg/mL)
3000
2000
1000
0
*
*
unstimulated
no additive
+TTA 100μM
+TTA 500μM
+BSA 100μM
+BSA 500μM
TNFα stimulated

FIG 3

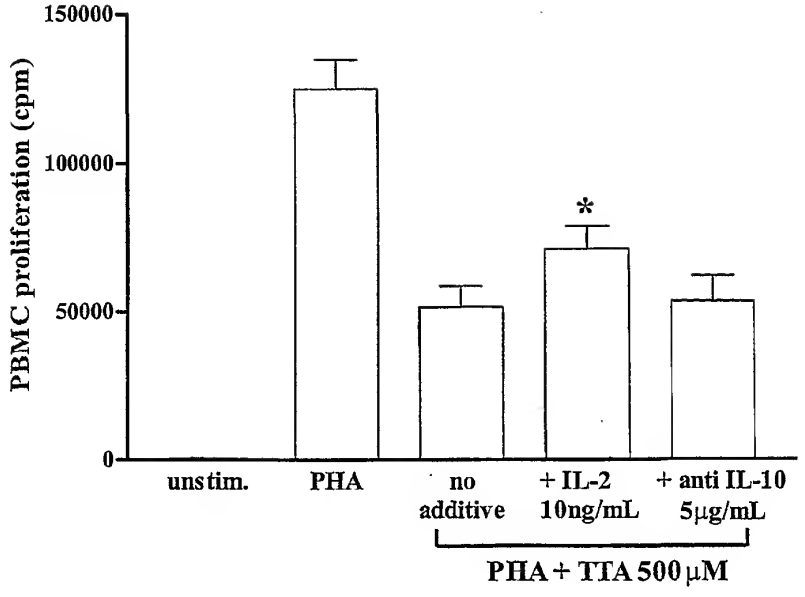
150000
100000
50000
0
PBMC proliferation (cpm)
*
unstim.
PHA
no additive
+ IL-2 10ng/mL
+ anti IL-10 5μg/mL
PHA + TTA 500 μM

FIG 4

INTERNATIONAL SEARCH REPORT

International application No.
PCT/NO 01/00470

A. CLASSIFICATION OF SUBJECT MATTER

IPC7: A61K 31/19, A61K 31/20, A61K 31/22, A61P 29/00, A61P 37/00
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC7: A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

SE,DK,FI,NO classes as above

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 0121575 A1 (WOMEN´S AND CHILDREN´S HOSPITAL ADELAIDE), 29 March 2001 (29.03.01) --	1-17
X	WO 9738688 A1 (PEPTIDE TECHNOLOGY PTY, LIMITED), 23 October 1997 (23.10.97) --	1-17
X	WO 9611908 A1 (PEPTIDE TECHNOLOGY LIMTED), 25 April 1996 (25.04.96) --	1-15
X	US 5151534 A (SHROOT ET AL), 29 Sept 1992 (29.09.92) --	1-15

[X] Further documents are listed in the continuation of Box C. [X] See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance: the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance: the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
3 April 2002	05 -04- 2002
Name and mailing address of the ISA/ Swedish Patent Office Box 5055, S-102 42 STOCKHOLM Facsimile No. +46 8 666 02 86	Authorized officer VIVECA NORÉN/BS Telephone No. +46 8 782 25 00

Form PCT/ISA/210 (second sheet) (July 1998)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/NO 01/00470

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 9900116 A2 (YISSUM RESEARCH DEVELOPMENT COMPANY OF THE HEBREW UNIVERSITY OF JERUSALEM), 7 January 1999 (07.01.99) --	1-17
X	Breast Cancer Research and Treatment, Volume 45, 1997, Farzaad Abdi-Dezfuli et al: "Eicosapentaenoic acid and sulphur substituted fatty acid analogues inhibit the proliferation of human breast cancer cell in culture", pages 229-239 --	16-17
A	WO 9958120 A1 (BERGE, ROLF), 18 November 1999 (18.11.99) --	1-17
A	DE 4120917 A1 (BASF AG), 7 January 1993 (07.01.93) --	1-15
A	WO 9412466 A1 (YISSUM RESEARCH DEVELOPMENT COMPANY OF THE HEBREW UNIVERSITY OF JERUSALEM), 9 June 1994 (09.06.94) --	1-15

INTERNATIONAL SEARCH REPORT

International application No.
PCT/NO01/00470

Box I **Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)**

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.: 7-15
because they relate to subject matter not required to be searched by this Authority, namely:

see next sheet

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II **Observations where unity of invention is lacking (Continuation of item 2 of first sheet)**

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.

Claims 7-15 relate to methods of treatment of the human or animal body by surgery or by therapy/ diagnostic methods practised on the human or animal body/Rule 39.1.(iv). Nevertheless, a search has been executed for these claims. The search has been based on the alleged effects of the compounds/compositions.

INTERNATIONAL SEARCH REPORT

Information on patent family members

28/01/02

International application No.
PCT/NO 01/00470

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
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INTERNATIONAL SEARCH REPORT

Information on patent family members

28/01/02

International application No.
PCT/NO 01/00470

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